Is there a genomic fingerprint of Radon-induced lung cancer? Comparison of genomic alterations in lung cancer specimens from high and low Rn zones.

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Abstract # 1572

Background

Rn-222 (Rn) is a radioactive gas found in rock and soil that can build up to dangerous levels indoors. It emits alpha particles that cause dsDNA breaks, increasing potential for carcinogenesis. Rn is the 2nd leading cause of lung cancer in the US after smoking, with Environmental Protection Agency estimates >15,000 deaths/yr from Rn. The EPA recommends Rn mitigation in homes with Rn levels ≥ 4 pCi/L. One in 4 homes in Rhode Island state, contain Rn ≥4.0 pCi/L, which is higher than the national average of 1 in 15 homes. In our pilot study, we performed a retrospective analysis of NGS assays of a small cohort of 159 advanced lung cancer (LC) patients from Lifespan Cancer Institute in RI, in which we noted more frequent mutations in two particular DNA repair genes and significant difference in the frequency of mutations in the DNA repair pathway. In this study, we aim to validate our findings in a larger national cohort based on CARIS tumor NGS assay, comparing data from higher Rn states to low Rn states. We hypothesize that the impact of Rn exposure may be reflected in lung cancer gene mutation (mut) profiles.

Methods

- Using commercial NGS assays, genomic DNA alterations in FFPE specimens from 159 lung cancer patients were retrospectively analyzed from Lifespan Cancer Institute in RI (2014-2019).
- Per the EPA, indoor Rn level ≥ 4 pCi/L is categorized as Zone 1; advised as an "action level" for Rn mitigation. Levels 2-4 pCi/L as Zone 2 and <2 pCi/L as Zone 3. Based on EPA Rn maps, we identified counties in 9 states (ID, KY, MT, OH, PA, SD, FL, AZ, MS) with high indoor Rn levels (≥ 4 pCi/L; HR), compared the gene mutation patterns in lung cancer cases of those residing in low Rn areas (<4 pCi/L; LR). Patients were categorized into HR and LR, based on their zip code of residence.
- The validation cohort consisted of 5532 lung cancer patients (Caris Life Sciences). Molecular profiles were obtained using NGS 592 gene panel.
- We assumed uniform tobacco smoke exposure across both the cohorts.
- Gene fusion detection by WTS was performed on mRNA isolated from a FFPE tumor sample using Illumina NovaSeq platform (Illumina Inc.) and Agilent SureSelect Human All Exon V7 bait panel (Agilent Technologies). Gene fusion by ArcherDx fusion assay (Archer FusionPlex Solid Tm panel) was detected by anchored multiplex PCR for targeted RNA sequencing.
- Genomic alterations were then classified into major pathways implicated in lung carcinogenesis largely based on the TCGA Pan Cancer Atlas.
- TMB was measured from 592 genes (1.4 megabases [MB] sequenced per tumor) by counting all nonsynonymous missense mutations/per tumor (excluding any known germline alterations). TMB-high was defined as ≥10 mutations/MB.
- In the validation cohort, p values adjusted for multiple comparison (q) of < 0.05 were considered significant. Statistical analysis of TMB values were compared using Wilcoxon Rank Sum.

Results

In the pilot cohort, 35 pts (22%) were in HR and 124 (78%) in LR zones. Adenocarcinoma histology was most frequent (73%) and smoking prevalence was high (75%) in both groups. Most prevalent alterations were *TP53, KRAS* and *CDKN2A* muts. In the HR, we noted more frequent recurrent muts in 2 DNA repair genes: *ATM* (11 vs 1%, p = 0.00086) and *CHEK2* (6 vs 0%, p = 0.047) when compared to LR group. When classified into major pathways implicated in lung carcinogenesis, higher frequency of mutations were seen in DDR in HR zones vs. LR (29 vs 13%, p 0.038).

In the validation cohort, 1433 (26%) pts were in HR and 4099 (74%) in LR zones (Table 1). *ATM* muts in HR group were more frequent (4.7 vs 3.4% in LR, p = 0.03) as well as *PALB2* (0.9 vs 0.4%, p = 0.02) while no difference seen in *CHEK2* (Fig 1). Other genes with significantly higher prevalence in HR were *TP53, SMARCA4 and NFE2L2* (q < 0.05); while *KMT2D, KEAP1, CDKN2A, MET, NF2, DNMT3A, CCND1 and FAS* show a trend (p < 0.05). *EGFR* muts were significantly more frequent in LR zones (8.4 vs 14.6%, q = 0.001). Similar to the pilot cohort, DNA repair pathway alterations trend to be higher in HR zones (14 vs 12%, p = 0.05) (Fig 4).

Table 1: Demographic characteristics

	High Radon Zone	Low Radon Zone
Total no. of patients (n)	**1433**	**4099**
Gender (n, %)		
Males	**702 (49)**	**2036 (50)**
Females	**731 (51)**	**2063 (50)**
Age (yrs)		
Average Age	**67.4**	**69.1**
Average Age Male	**67.3**	**69.2**
Average Age Females	**67.5**	**69**
Histology (n, %)		
Adenocarcinoma	**876 (61)**	**2701 (66)**
Squamous	**320 (22)**	**742 (18)**
Other histologies	**237 (17)**	**656 (16)**

Figure 1: Most frequent mutations for HR vs LR. *TP53, SMARCA4, NFE2L2* all show significantly higher in HR than in LR zones. *EGFR* showed significantly lower in HR than in LR zones. Connective lines indicate statistical significance (q<0.05)

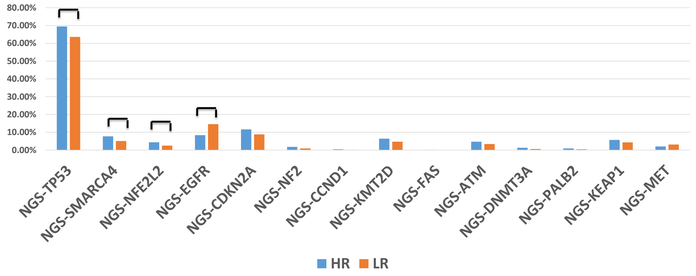


Figure 2: Gene amplifications showing a trend of being different in HR and LR zones (p <0.05 and q>0.05).

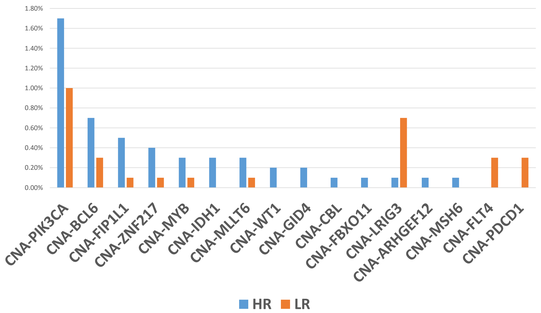
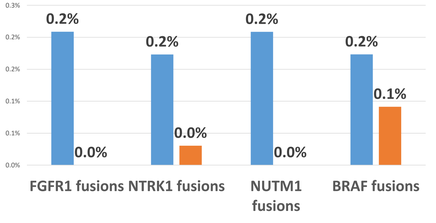


Figure 3: Total fusions observed in HR vs LR zones. Fusions were tested by either WTS or Archer. No statistical significance observed



Test	Positive	Negative	Total	HR	Positive	Negative	Total	LR	p value
FGFR1 fusions	2	956	958	0.2%	0	2639	2639	0.0%	ns
NTRK1 fusions	2	1153	1155	0.2%	1	3287	3288	0.0%	ns
NUTM1 fusions	2	957	959	0.2%	0	2639	2639	0.0%	ns
BRAF fusions	2	1152	1154	0.2%	3	3283	3286	0.1%	ns

Using a high TMB cut-off ≥10, tumors from HR zones had significantly higher TMB when compared to LR zones (56 vs 48%, q = 0.0005) (Fig 5). On sub-group analysis, only adenocarcinoma histology had significantly higher TMB in the HR zones (p<0.001). Median TMB in HR zones is 12.1 vs 11.5 in LR zones (p<0.001).

Figure 4: Pathway analysis for HR vs LR. *TP53*, Chromatin Remodeling, RTK and KEAP1/NRF2 were significantly different between HR vs LR while Cell cycle and DDR pathways were trending. Connective lines indicate statistical significance (q<0.05)

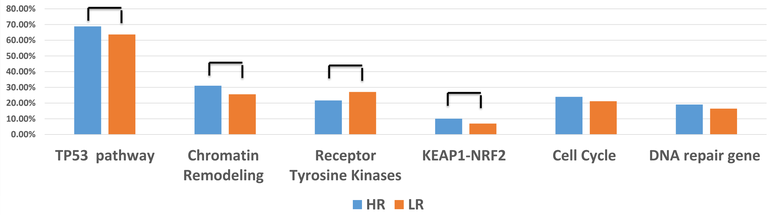
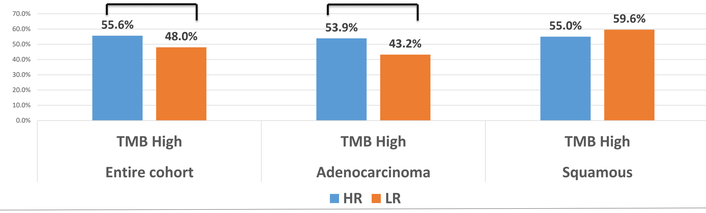


Figure 5: TMB-High in radon zones. TMB high was significantly higher in the high radon zone (p<0.001). In adenocarcinoma, TMB high was significantly higher in the high radon zone as well (p<0.001). Connective lines indicate statistical significance (q<0.05)



Conclusions

- To our knowledge, this is the first attempt to elucidate the pathobiology of Rn induced lung cancer using gene mutation analyses. Our observations suggest that high Rn exposure induces dsDNA breaks, which constitutes an oncogenic hit in cells which are unable to efficiently repair them because of defective DNA repair pathway.
- Lung cancers from high radon zones overall demonstrate a significantly higher TMB than in low radon zones, particularly in adenocarcinoma histology.
- TP53, Chromatin Remodeling and KEAP1-NRF2 pathways were significantly higher in high radon zones where Receptor Tyrosine Kinase pathway was significantly lower in high radon zones.
- Assuming uniform tobacco smoke exposure, higher Rn was not associated with *EGFR* mut.

References

1. Basic Radon Facts. EPA. July 2016. https://www.epa.gov/sites/production/files/2016-08/ documents /july_2016_radon_factsheet.pdf
2. Pan-Cancer Atlas. https://www.cell.com/pb-assets/consortium/pancanceratlas/pancani3/index.html

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